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Research Review

Changes Encountered in Tea [*Camellia sinensis* (L.) Kuntze] in Response to Sucking Pest like Red Spider Mite and Defensive Strategies Involved – A Mini Review

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Abstract

Red spider mite is one of the major sucking pests of different tea growing countries that damage the tea leaves resulting in crop loss as well as deteriorating the quality of made tea severely. Changes encountered in physiological and biochemical constituents in green leaves of tea in relation to photosynthetic pigments, wax content, effect on phytohormonal levels of abscisic acid, jasmonic acid etc., and their effect on the production of secondary metabolites as well as on the quality of made tea was studied in detail. Pigments like chlorophyll a and b, carotenoids and non-volatile secondary metabolic compounds such as polyphenols, catechins and reducing sugar were found to be lower in the severely infested leaves than the non-infested leaves. Morphological defense strategy includes the increased trichomes, sclerophylly, latex deposition, etc. while biochemical defences include production of various toxic secondary metabolites, some enzymes that act as pest-repellents. Plants are constantly subjected to biotic stress therefore plants make necessary metabolic and structural modifications to cope with the stress conditions. As tea is an economically important beverage, a proper understanding of the plant-insect interaction would facilitate better pest management and quality production. Defence mechanisms adopted therefore strengthens the plant to deal with multiple biotic as well as abiotic challenges.

***Key words*:** Red spider mite, Physio-biochemical changes, Defence response, Pest, Stress related enzymes

Tea which is a perennial plant, grows more than 100 years and is subjected to the attack of insects, mites, nematodes and some plant pathogenic funguses in its lifetime. Insects in general are of great importance and helps in pollination, seed dispersal and protection from other herbivores but at times it reaches to a level where the host plant is severely damaged and can destroy the entire plant. Tea is the most preferred health beverage in the international market made from the leaves of evergreen shrub Tea (*Camellia sinensis* (L.) Kuntze) belonging to the family Theaceae. It is healthy for human consumption due to the presence of beneficial metabolites [1]. Presently tea is grown in more than 50 countries round the globe and is found in plains and hills up to 2300m altitude. The top producers of tea are China, India, Sri Lanka, and Kenya. Vietnam, turkey, Indonesia, Argentina, Japan, Bangladesh, Malawi, African nation and United Republic of Tanzania manufacture the remaining of the world tea crop. Several species of tea mosquito bugs, spider mites and loopers cause major damage in tea plantations for which every year the industry faces huge crop losses because of these pests whereas thrips, aphids, jassids, flush worms and nematodes are the predominant pests in nurseries and young tea plantations [2]. Insect herbivory is the significant factor that causes 11-55% loss in tea production every year [3-4] and also reduces crop quality [5]. Sometimes continuous feeding may cause reduced plant growth and defoliation leading to sustained production loss and deterioration of quality. The pests that attack tea bushes cause severe damage thereby bringing about changes in terms of physio-biochemical properties in green leaves as well as the quality of made tea. They are divided into two categories according to their feeding mechanism: chewing pests and piercing-sucking pests [6]. Damage done by sucking pests like thrips and mites led to dull appearance of tea in the fields. When black tea production is done with such highly pest infested leaves it significantly affected flavor and reduced the polyphenolic content [7-8].

With the process of evolution, plants have developed various defensive strategies to fight and prevent the damage from attack of pests by producing some physical / morphological and biochemical defenses. Morphological

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defense strategy includes the increased quantity of trichomes [9], sclerophylly, latex deposition, etc. while biochemical defences include production of various toxic secondary metabolites, some toxic non-metabolites and enzymes acting as pest-repellents. During attack of pests, a surge in accumulation of reactive oxygen species (ROS), and phytohormones like jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), and ethylene (ET) is seen which play a key role in induced defence system [10-11]. Variation in cytosolic Ca^{2+} ion levels and H_2O_2 formation and rapid changes in plasma membrane potential are the introductory events of plant-pest interaction [12]. Different biochemical changes which are induced in plants due to pest attack are found and discussed in (Fig 1).

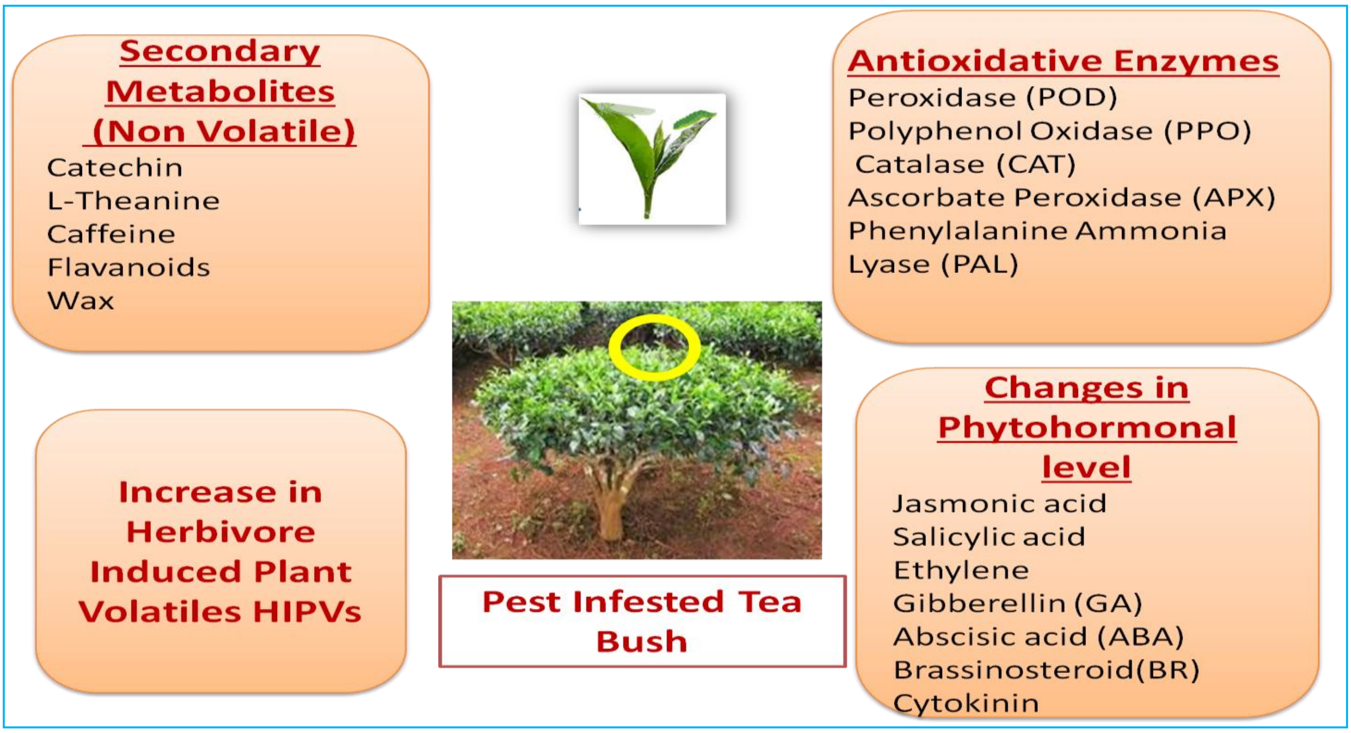

Fig 1 Biochemical changes induced due to pest infestation in tea

ABA, abscisic acid; APX, ascorbate peroxidase; BR, brassinosteroid; CAT, aatalase; ET, ethylene; HIPVs, herbivore induced plant volatiles; JA, jasmonic acid; PAL,phenylalanine ammonia lyase; POD, peroxidase; PPO, polyphenol oxidase; SA, salicylic acid; SOD, superoxide dismutase

Red spider mite: *Oligonychus coffeae* Neitner

Oligonychus coffeae is one of the important and serious pests found in tea plantations across the globe that results in reduced yield and degraded quality of tea incurring serious economic loss. *Oligonychus coffeae* shows great fecundability and multiplies at an alarming rate due to its short life cycle and is a major constraint within the tea industry and they are the most difficult to manage. They are found on the dorsal and ventral surface of mature tea leaves, along with thousands of eggs [13]. Mostly all the stages including the larvae, nymphs and adults (Fig 2) causes damage to the plant. Infestation begins mainly along the midrib, veins and then gradually covers the entire upper surface of leaves. They feed on the leaves, thereby damaging their potential to photosynthesize. The maintenance foliage of the bush turns reddish bronze in colour, making red it distinct clear even from a far distance (Fig 3). Severe infestation by this mite ultimately leads to defoliation [14]. Red spider mite is one of the important mite pests of tea causing damage in respect to both quantity and quality.

Fig 2 An adult mite under bright-field microscope (Labomed LX300) at 10X

Fig 3 Severe infestation of a mature bush with red spider mite

This review is aimed to throw light on the response of the plant in relation to red spider mite infestation in the green leaves as well as in manufactured tea. Studies clearly showed that the physiological and biochemical contents were significantly decreased with the increase in severity of the infestation of red spider mites which is shown in (Fig 4). Pigments responsible for carrying out photosynthetic activity like chlorophyll a and b, carotenoids and non-volatile secondary metabolic compounds such as polyphenols, catechins and reducing sugar in severely infested green leaves were found to be lower than the non-infested fresh leaves [15]. Made tea prepared from severely infested leaves had lower amount of theaflavin (TF), high polymerized substances, total liquor colour, colour index, lipid, caffeine, moisture content and water extract but it is reverse in case of thearubigin (TR), total ash and dry matter.

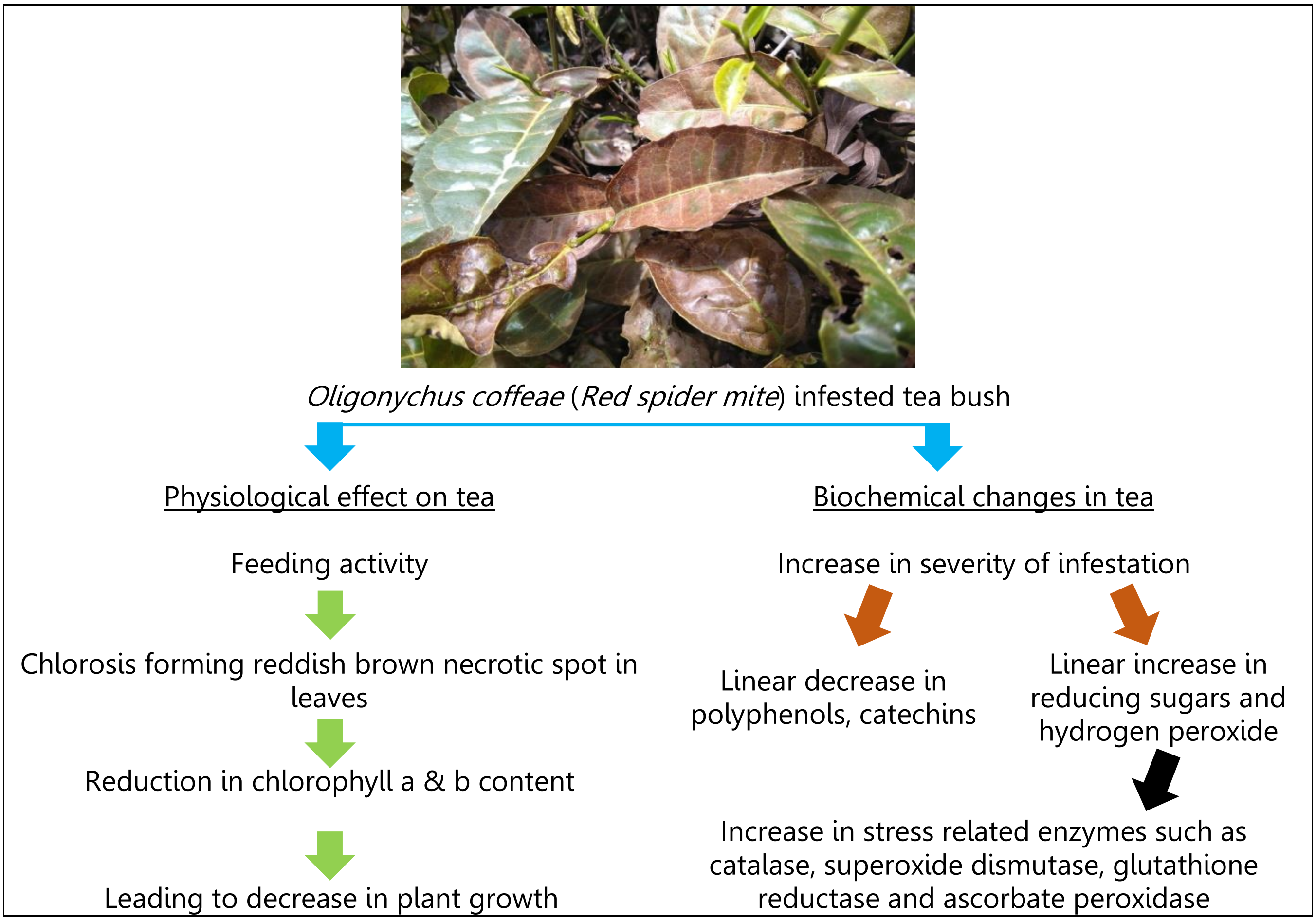

Fig 4 Flow diagram clearly depicting that the physiological and biochemical changes observed in tea bushes with severe infestation by red spider mites

Organoleptic evaluation also confirms that tea made from such mite infested leaves were flat in taste due to the presence of excreta of the larvae thereby making it poor quality [16]. Therefore, it can be said that results of organoleptic evaluation correspond with the analytical results done in laboratory. Tea is a revenue earning crop for the country and therefore the damage caused by red spider mite is considered as the major setback in tea industry causing both qualitative and quantitative damage and is a matter of serious concern. Crop loss due to mite infestation was assessed in different regions across the world [17-19]. Less information on the physiological and biochemical changes as well as quality deterioration of made tea caused by the infestation of red spider mite so further research need to be done in this line.

Physiological changes in relation to chlorophyll content and photosynthetic efficiency

Infestation by *Oligonychus coffeae* on the upper surface of the leaves of *Clonorchis sinensis*, near the midrib or veins, was previously mentioned by [20]. The observations showed that mite infestation was highest on mature leaves as reported by [21]. Under microscopic observation, the infested leaves showed the presence of large number of reddish-brown spots due to the feeding activity of the mite. The feeding punctures were often found coalesced to form bronzy areas. Severe infestation by red spider mite showed symptoms of prominent chlorosis in the leaves forming necrotic lesions on the laminar surface of leaf. Estimation of chlorophyll content revealed drastic reduction in both Chorophyll a and Chlorophyll b contents in the heavily infested leaves. Chlorophyll a in heavily infested leaves showed a sharp decline and similarly the reduction in chlorophyll b was also clear when compared with the chlorophyll content of non-infested leaves in (Fig 5). It plays an essential role in the photosynthetic process thereby any change in its content is directly reflected the photosynthetic efficiency and assimilation capacity. Physiological parameters such as photosynthetic rate, stomatal conductance, water use efficiency and chlorophyll fluorescence were reduced in the infected leaves. The efficiency of green leaves to capture light during photosynthesis is directly correlated to the chlorophyll concentration in the leaf [22]. The heavy loss of chlorophyll content as evidenced by the present study revealed the potential of *Oligonychus coffeae* to affect harmfully the general health of host plant, *Clonorchis sinensis*, thereby leading to decrease in the growth rate and biomass of the plant.

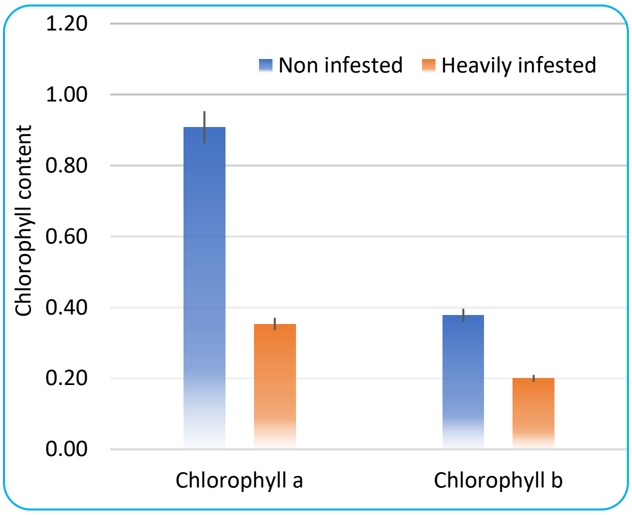

Fig 5 Reduction in chlorophyll content in heavily infested bush when compared with the non-infested bush [23]

Biochemical changes in green leaf and made tea

Interactions between activities of enzymes and clones with respect to RSM damage were elucidated in this review. Although there are some reports on bush physiology but detailed work on changes in biochemical constituents including enzymes is less. Shoots collected were grouped into healthy, moderately infested and severely infested and were used for determining the biochemical constituents and quality. Stress-related enzymes like superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase were studied. It was observed that with severe damage caused by mites led to a sharp decline in Polyphenols, catechins and chlorophyll content while reducing sugars and hydrogen peroxide increased in all clones. Initially quality related enzymes were enhanced due to RSM attack but later showed a sharp decline when the infestation was severe. Therefore, it can be concluded that all stress related enzymes linearly increased with the increase in severity of RSM infestation.

Correlation between physical barriers like trichome density and pest attack

With the formation of a waxy cuticle, development of spines, setae, and trichomes the first line of plant defence mechanism is created against insect pests that acts as a physical barrier. Structural defences include morphological and anatomical modifications that render resistance to the plant by directly deterring the herbivores from feeding, and range from prominent perturbances on a plant to thickened cell wall as a result of deposition of lignin. Leaf thickness or toughness was found to be greatest in TV6 followed by TV10 and TV1 when compared among these three clones [24]. Thickened leaf provide greater resistance as less feeding was observed in thick TV6 leaves than thin and moderate TV1 and TV10 leaves. The waxy cuticular layer of TV1 is comparatively thin enough to pierce hence maximum damage by *O. coffeae* is observed which confirms the findings of Hanna *et al*. [25] and leaves with thin cuticle are infested more easily by tetranychids. Therefore, it can be concluded that thickness of leaf may reduce the incidence of *O. coffeae* attack. Trichomes play an important role in plant defense against many insect pests and involve both toxic and deterrent effects. Trichome density is negatively correlated with the ovipositional behavior, feeding and larval nutrition of insect pests. Moreover, thick and dense trichomes affect the herbivory mechanically by providing a physical barrier and hinder the movement of insects on the plant surface. Structurally trichomes can be of diverse types including straight, spiral, hooked, branched, or unbranched and can be glandular or non-glandular. Glandular trichomes secrete certain bioactive non-volatile compounds like flavonoids, terpenoids, and alkaloids which can be poisonous, repellent, or trap insects and other organisms, thus forming a combination of structural and chemical defense. Induction of trichomes in response to pest damage has been reported in many plants. The change in trichome density in response to damage by insects can only be observed in leaves developing during or subsequent to insect attack, as the density of trichomes of existing leaves remain unchanged. Trichomes form the first line of defense against insect pests, and play an important role in host plant resistance. Sclerophylly refers to the hardened leaves, and plays an active role in plant defence against herbivores by reducing the palatability and digestibility of the tissues, thereby, reducing the herbivore damage. The increase in trichome density in response to herbivory is typically between 25-100%. Changes in trichome density generally occurring within days or weeks after insect damage. Hence a positive correlation can be drawn between natural enemies' abundance and trichome density.

Role of non-volatile secondary metabolites in Camellia involved in defence response

In tea, certain specialized non-volatile secondary metabolites attribute to the health benefits, flavour and unique taste of tea which include amino acids (L-theanine), polyphenols (catechins), caffeine (methylxanthines), and used as important parameters for quality estimation [26]. Different biotic and abiotic factors affect the levels of these specialized metabolites. Different research groups have already confirmed that the accumulation of volatile secondary metabolites is a common phenomenon in tea plant due to insect herbivory, but subsequently recent studies have demonstrated that some non-volatile metabolites were also found due to infestation of chewing and piercing-sucking pests. A recent study demonstrated increased theaflavin from catechin, L-theanine concentration, but caffeine content was not significantly changed [27] which is shown in (Fig 6).

Level of different flavonoid compounds like flavone glycosides, flavonol glycosides, proanthocyanidin, and tannins were increased during insect infestation while catechins were elevated occasionally [28] although a study by Liao *et al*. [29] demonstrated no significant change of catechin level. Flavonoids protect plants against insect pests via degrading nutritive value, altering plant palatability, or may act as toxins that act as insect repellants [30]. It was found that attack by piercing-sucking pest like mites and tea mosquito bugs and chewing pests like loopers, the catechin content was low but the catechin oxidation product theaflavin (TF) was enhanced due to activation of PPO enzyme during piercing-sucking pest infestation while there was no significant effect by chewing pest infestation [31]. Although there is a thought that catechins play a defensive role, but it is not fully proved.

Increase in L-theanine was also observed during infestation of pests. The enzyme L-theanine synthase (TS) triggers the biosynthesis of L-theanine from ethylamine and L-glutamic acid. As there is a steady level of the ethylamine maintained all time therefore Liao *et al*. [31] concluded that insect attack induced L-theanine might be due to some unknown factors.

Caffeine is the major among the tea alkaloids which is not significantly changed by pest attack but its key precursor compounds such as S-adenosyl-L-methionine synthase and caffeine synthase are affected [31-32]. Cuticle is a thin waxy outermost layer present on the epidermal surface of leaves that acts as a protective shield against moisture loss and abiotic stress, and is involved in defending against microbial pathogens and herbivore insects. The cuticular wax alkane formation was significantly upregulated and C29 alkane level was increased. C29 alkane is responsible for wax deposition in tea leaf [28].

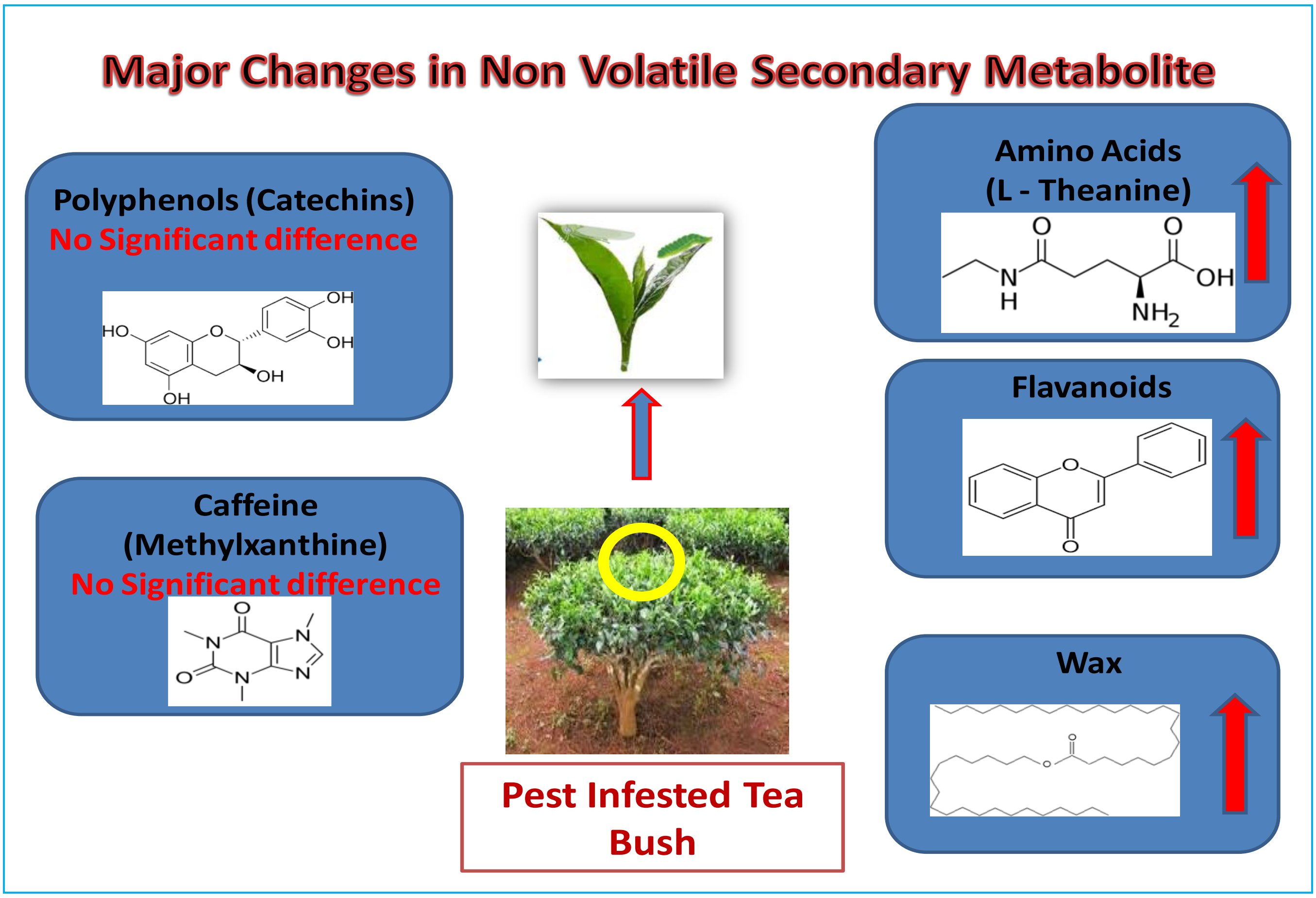

Fig 6 Changes in different non-volatile secondary metabolites induced by pest infestation in tea

Role of phytohormones in defense response in plants

Extensive research on plant defense study shows that JA is mainly engaged in the initiation of defensive system in response to herbivorous insect attack while salicylic acid is induced against pathogens [33]. The major plant growth regulators like Jasmonic acid, Salicylic acid and Ethylene safeguard the plant defense system, while gibberellin (GA), abscissic acid (ABA), brassinosteroid (BR), and cytokinin are additionally included at times in plant-insect interaction [34]. The signal transduction pathways triggered by JA and SA is responsible for defense mechanism [35]. The Jasmonic acid signal transduction pathway is playing the most important role in the induction of host induced plant volatiles HIPV [36]. In tea plants JA and SA get enhanced both by chewing and piercing-sucking pests but ABA increases only in case of attack by chewing pests. Inter-relationship of phytohormones and non-volatile metabolites was determined by Liao *et al.* [31]. They found that Abscisic acid affected theaflavin, SA affected caffeine, and both JA and SA affected catechins. They reported that the genes tea caffeine synthase1 (TCS1) and S-adenosylmethionine synthase 1 (CsSAM1), involved in the biosynthesis of catechins were significantly increased by SA, contrary to the volatile compounds. Phytohormones did not play a major role in changing the characteristic non-volatile metabolites.

Caffeine induced increase in JA levels is attributed to JA biosynthesis through the lipoxygenase (LOX) pathway in tea leaves [37]. Induction of transcripts associated with jasmonate and ethylene production was due to infestation of chewing insect [38]. Activity of JA synthesis genes such as linoleate 13S-lipoxygenase2-1, omega-3 fatty acid desaturase, and jasmonate O-methyltransferase were significantly increased during pest attacks [39-40]. Induction pathways elicited by JA are involved in synthesizing most important secondary metabolites like flavonoids, alkaloids, and terpenoids [41]. Ethylene and JA signaling pathways sometimes work together either synergistically or antagonistically during insect infestation [42].

Enzymes responsible for pest induced defense mechanism in plants

Tolerance mechanism in *Camellia* is attributed to the several enzymatic changes which takes place as a result of biotic stress induced by insect herbivory. The damage caused in the leaf tissue leads to the accumulation of ROS. Reactive oxygen species (ROS) produced in plants are detoxified by defense responsive enzymes like peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), ascorbate peroxidase (APX), and catalase (CAT) [43]. Catalase plays a significant role in detoxification of H_2O_2, so when catalase activity gets reduced, it is unable to detoxify the H_2O_2, hence resulting in oxidative damage (Bi et al. 1997). During infestation of a tea plant by sucking pests the level of polyphenol oxidase, peroxidase, phenylalanine ammonia lyase and ascorbate peroxidase increases, while the level of catalase decreases [43]. According to Yang *et al.* [44] an increase in PPO, POD, and PAL could inhibit larval growth.

In pest-induced defense response of plant, H_2O_2 plays the central role among all ROS. Redox cycling of quinone lead to the production of H_2O_2 and other ROS. H_2O_2 acts as a substrate of peroxidase enzyme so eventually when the plant faces insect attacks the substrate availability increases through a cascade of metabolic events, which may consequently increase the peroxidase (POD) level. Pest resistance activity conferred by the enzyme polyphenol oxidase (PPO) is confirmed by several authors. PPO increased the cell wall resistance to insect [45]. Oxidative changes like H_2O_2 accumulation in plant cause damage in insect midgut which also triggers many physiological and molecular responses in the host plant against insect attacks, and maintains an elevated level of H_2O_2, till the herbivore attack persists [46-47].

Phenylalanine ammonia lyase (PAL) is one of the important enzymes and is responsible for the synthesis of various phenolic compounds involved in defense mechanism of plant pathogen [48]. PAL is a key enzyme controlling the biosynthesis of different metabolites, such as flavonoids, phenylpropanoids and lignin in plants [49]. These enzymes basically interfere with the nutrient uptake and metabolism of herbivore pests from the host plant.

Plants release numerous volatile compounds upon insect and pathogen attacks. The volatile compounds not only impart characteristic aroma to tea as a quality parameter but also serve as defense signals and help in cross talk for plant-insect interactions [28]. Immediate response of plants to damage, in most cases is the buildup of oxidative enzymes required for scavenging toxic radicals [50]. It was found that all oxidative enzymes peroxidase, ascorbate peroxidase and polyphenol oxidase, increased following insect damage, whereas phenyl alanine ammonia lyase, a key enzyme in the phenol biosynthetic pathway, decreased. Wounding by pests may have been one of the primary inducers of the oxidative enzymes. Oxidative enzymes like polyphenol oxidase and peroxidase, as well as the ones involved in phenolic biosynthesis like phenyl alanine ammonia lyase and tyrosine ammonia lyase, are responsible for defense reaction in plants [51]. Several studies have confirmed the importance of phenolic compounds in plant defense. Plant phenolics generally have a diverse range of biological activity, depending on their structure, degree of polymerization, stereoisomeric differences, etc. It can be concluded that total phenolic contents decreased with insect attack. Catechins is a class of polyphenols, that comprises major phenolic components of tea leaves. HPLC analysis revealed gallocatechin gallate to be predominant, followed by catechin gallate [52]. Pest attack reduced both gallocatechin gallate GCG and catechin gallate CG, while a slight increase in epicatechin was observed. Theaflavins, which is the oxidized product of catechins formed by the action of polyphenol oxidase, is the prime factor for quality assessment in tea. No significant difference was found between the theaflavins observed in healthy and insect damaged leaves, which may be due to the increased activity of PPO during insect damage. This indicates that insect attack did not affect the flavor component, but was responsible only for a reduction in biomass [52]. One of the reasons for the tolerance of certain varieties to insect attack could be their ability to maintain higher levels of phenolics during the attack.

CONCLUSION

Plants are constantly subjected to different stresses due to attack by pests and pathogens therefore plants make necessary metabolic and structural modifications to cope with the stress conditions. This is a nature-based solution to address critical ecological challenges at multiple scales. Induction of plant defense response by pests, insects and other pathogens involve a large area of research and study. Much advance study needs to be undertaken in tea plants specially in the field of different secondary metabolites, their functions and pharmacological properties, their potentiality in preventing attack by pests and pathogens. Conclusion can be drawn that the infestation of red spider mites in tea bushes not only affects the physiological and biochemical contents of green leaves but also on the quality of made tea. This review collates the work and literature on the different metabolites and other less known chemicals released by the tea plant, their biosynthesis pathway, and effects of herbivory by insects. Many volatiles and non-volatile compounds are identified in tea plants but no detailed studies have been done regarding their involvement in defense mechanisms. The defence mechanisms adopted by the tea plant strengthens the capacity of the plant to deal with multiple biotic as well as abiotic challenges, which itself is a nature-based solution. As tea is one of the beverage plants of great economic value, a proper understanding of the plant-insect interaction would facilitate better pest management and quality production. Great challenges are faced while trying to develop ingenious alternative methods to prevent attack of insect pests with economic suitability and environmental sustainability. Presently the control of the tea pest is mainly dependent on chemical pesticides, which has given rise to the potential problems associated with the development of pesticide resistance. Hence, bio active phytochemicals are recommended by many researchers to overcome this problem. Biodegradable compounds can be used to replace hazardous chemical pesticides and it will be an important step towards the environmentally sustainable pest management. Induction and maintenance of different secondary metabolites like terpenoids, alkaloids and phenolic compounds in plant system may become alternative option for host defense as compared to traditional chemical pesticides. With extensive research efforts on host defense metabolites environmentally sustainable pest management strategies may be developed in future. As tea consumption is gaining popularity day by day, all over the world, due to widespread campaigning on its health benefits. Hence, further research is required in this line to minimize the loss in green leaves due to red spider mites infesting tea. Most of the bioactive compounds likes polyphenols, caffeine, reducing sugars, total antioxidants, tannins, pigments like chlorophyll, ash, colour index, etc. were found to be much lower in the mite infested leaves in comparison to the fresh leaves. By the attack of red spider mite, the production of tea leaves is also reduced and consequently reduces the total crop. Thus, knowing the behaviour of mites under variable conditions may be helpful in rescheduling the miticides use and modifications of some available control options to reduce the infestation of red spider mites in tea. So, further research is needed to control the invasion of red spider mite in tea gardens to safeguard the quality and sustainable production of tea.

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Declaration of competing interest

The authors declare that there is no conflict of interests.

Credit authorship contribution statement

S.R. and C.G. conceived this study. S.R performed data extraction and analysis. S.R drafted the manuscript. P.K.P. commented, reviewed and upgraded the manuscript with photographs. All the authors equally contributed in writing results and discussions. C.G. revised the manuscript thoroughly to give the manuscript a final shape and all authors agreed with the final version.

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